

CLAIMS

1. A non-hemolytic cytolytic agent selected from a peptide, a complex of bundled peptides, a mixture of peptides or a random peptide copolymer, said agent having a selective cytolytic activity manifested in that it has a cytolytic activity on pathogenic cells, being cells which are non-naturally occurring within the body consisting of microbial pathogenic organisms and malignant cells; and it is non-hemolytic, namely it has no cytolytic effect on red blood cells or has a cytolytic effect on red blood cells at concentrations which are substantially higher than that in which it manifests said cytolytic activity, said non-hemolytic cytolytic agent being selected from the group consisting of:

- (1) a cyclic derivative of a peptide having a net positive charge which is greater than +1, and comprising both L-amino acid residues and D-amino acid residues, or comprising one or both of L-amino acid residues and D-amino acid residues, and comprising an α -helix breaker moiety;
- (2) a peptide comprising both L-amino acid residues and D-amino acid residues, having a net positive charge which is greater than +1, and having a sequence of amino acids such that a corresponding amino acid sequence comprising only L-amino acid residues is not found in nature, and cyclic derivatives thereof;
- (3) a complex consisting of a plurality of 2 or more non-hemolytic cytolytic peptides, each peptide having a net positive charge which is greater than +1, and comprising both L-amino acid residues and D-amino acid residues, or comprising one or both of L-amino acid residues and D-amino acid residues and comprising an α -helix breaker moiety, or cyclic derivatives of the foregoing, said peptides being bundled together by the use of a linker molecule covalently bound to each of the peptides;
- (4) a mixture consisting of a plurality of 2 or more non-hemolytic cytolytic peptides, each peptide having a net positive charge which is greater than +1, and comprising both L-amino acid residues and D-amino acid residues, or comprising one or both of L-amino acid residues and D-amino acid residues and comprising an α -helix breaker moiety, or cyclic derivatives of the foregoing; and
- (5) a random copolymer consisting of different ratios of a hydrophobic, a positively charged and a D-amino acid.

2. The cyclic peptide according to claim 1(1), comprising both D- and L-amino acid residues having a sequence such that a homogeneous open-chain peptide comprising only L- or only D-amino acid residues and having the same amino acid sequence as said peptide, has an α -helix configuration and has a broad spectrum cytolytic activity manifested on a variety of cells.

3. The cyclic peptide according to claim 2, which is a cyclic diastereomer derived from pardaxin or mellitin or from fragments thereof.

4. The cyclic peptide according to claim 3, in which the net positive charge greater than +1 is due to the native amino acid composition, or is attained by neutralization of free carboxyl groups or by the addition of positively charged amino acid residues and/or positively charged chemical groups.

5. The cyclic peptide according to claim 4, which is selected from a cyclic diastereomer of pardaxin or of a fragment thereof to which Lys residues have been added to the N-terminus and/or aminoethylamino groups have been added to the C-terminus.

6. The cyclic peptide according to claim 5, selected from the cyclic pardaxin-derived peptides herein designated peptides 86-88, of the sequence: (SEQ NOS: 86-88, respectively)

86. Cyclic K¹[D]P⁷L¹⁸L¹⁹[1-22]-par of the sequence:

Cys-Lys-Gly-Phe-Phe-Ala-Leu-Ile-Pro-Lys-Ile-Ile-Ser-
Ser-Pro-Leu-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Cys

87. Cyclic K¹K²[D]P⁷L¹⁸L¹⁹[1-22]-par of the sequence:

Cys-Lys-Lys-Gly-Phe-Phe-Ala-Leu-Ile-Pro-Lys-Ile-Ile-Ser-
Ser-Pro-Leu-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Cys

88. Cyclic K¹K²K³[D]P⁷L¹⁸L¹⁹[1-22]-par of the sequence:

Cys-Lys-Lys-Lys-Gly-Phe-Phe-Ala-Leu-Ile-Pro-Lys-Ile-Ile-
Ser-Ser-Pro-Leu-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Cys

7. The peptide according to claim 1(2), comprising both L-amino acid residues and D-amino acid residues and having a sequence of amino acids such that a corresponding amino acid sequence comprising only L-amino acid residues is not found in nature.

8. The peptide according to claim 7, having the following characteristics:

(a) it is a non-natural synthetic peptide composed of varying ratios of at least one hydrophobic amino acid and at least one positively charged amino acid, and in which sequence at least one of the amino acid residues is a D-amino acid;

10 (b) the peptide has a net positive charge which is greater than +1; and

(c) the ratio of hydrophobic to positively charged amino acids is such that the peptide is cytolytic to pathogenic cells but does not cause cytolysis of red blood cells.

9. The peptide according to claim 8, wherein the positively charged amino acid is selected from lysine, arginine and histidine, and the hydrophobic amino acid is selected from leucine, isoleucine, glycine, alanine, valine, phenylalanine, proline, tyrosine and tryptophan.

10. The peptide according to claim 9, wherein the net positive charge greater than +1 is due to the amino acid composition or to the addition of positively charged chemical groups, or which hydrophobicity may be decreased by the addition of polar amino acids such as serine, threonine, methionine, asparagine, glutamine and cysteine.

11. The peptide according to claim 10 having at least 6 amino acid residues, in which the hydrophobic amino acid is leucine, alanine or valine, and the positively charged amino acid is lysine.

12. The peptide according to claim 11, being a diastereomer of a 6-mer, 8-mer or 12-mer peptide composed of leucine and lysine, in which at least one third of the sequence is composed of D-amino acids, but excepting the peptide herein designated 23₁ (SEQ ID NO. 23)

23. Lys-Leu-Leu-Leu-Lys-Leu-Leu-Leu-Lys-Leu-Leu-Lys-NH₂

13. A Leu/Lys diastereomer according to claim 12, selected from the peptides herein designated 24 to 29, (SEQ ID NO: 24-29, respectively) of the sequence:

24. Lys-Leu-Leu-Leu-Lys-Leu-Lys-Leu-Lys-Leu-Leu-Lys-NH₂
 25. Lys-Lys-Leu-Leu-Lys-Leu-Lys-Leu-Lys-Leu-Lys-Lys-NH₂
 26. Lvs-Leu-Leu-Leu-Lvs-Leu-Leu-Leu-Lvs-Leu-Leu-Lvs-NH₂
 27. Lvs-Leu-Leu-Leu-Lvs-Leu-Lvs-Leu-Lvs-Leu-Lvs-Leu-Leu-Lvs-NH₂
 28. Lys-Leu-Leu-Leu-Lys
 29. Lys-Leu-Leu-Leu-Lys-Leu-Leu-Lys

14. The cyclic derivative of a non-natural synthetic peptide according to ^{claim 7} ~~any one of~~ ~~claims 7-13~~, selected from the peptides herein designated 92-95, (SEQ ID NOS: 92-95, respectively) of the sequence:

92. Cyclic Cys Lys Leu Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Cys
 93. Cyclic Cys Lys Leu Leu Leu Lys Leu Lys Leu Lys Leu Leu Lys Cys
 94. HN - Lys Leu Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys - CO
 95. HN - Lys Leu Leu Leu Lys Leu Lys Leu Lys Leu Leu Lys - CO

15. A complex of bundled peptides according to claim 1(3) consisting of a plurality of 2 or more non-hemolytic cytolytic peptides according to ^{claim 1} ~~any one of claims 1-14~~, said peptides being bundled together through a linker molecule covalently bound to each of the peptides.

16. The complex according to claim 15, wherein the bundle is composed of 2 or more, preferably 5, molecules of the same peptide or of different peptides, and the linker is a peptide according to any one of the preceding claims or a commonly used linker.

17. The complex according to claim 16 selected from the bundled Lys/Leu diastereomers herein designated 96 and 97:

96. ([D]-L^{3,4,8,10}-K₄L₈C)₅ [D]-L^{3,4,8,10}-K₄L₈ of the sequence:

(Lys-Leu-Leu-Leu-Lys-Leu-Leu-Leu-Lys-Leu-Leu-Lys-Cys-NH₂)₅ Lys-Leu-Leu-Leu-Lys-Leu-Leu-Leu-Lys-Leu-Leu-Lys-NH₂ (SEQ ID NOS: 96 and 23)

97. ([D]-L^{3,4,8,10}-K₅L₇C)₅ [D]-L^{3,4,8,10}-K₄L₉ of the sequence:

(Lys-Leu-Leu-Leu-Lys-Leu-Lys-Leu-Lys-Leu-Leu-Lys-Cys-NH₂)₅ Lys-Leu-Leu-Leu-Lys-Leu-Leu-Leu-Lys-Leu-Leu-Lys-NH₂ (See ID nos: 92 and 24)

18. The mixture according to claim 1(4) consisting of a plurality of 2 or more non-hemolytic cytolytic peptides, wherein the peptides are as defined in ^{claim 1} ~~any one of claims 1 to 14~~.

19. The mixture according to claim 18 comprising a mixture of Lys/Leu 12-mer peptide diastereomers.

20. The non-hemolytic cytolytic random copolymer according to claim 1(5), consisting of different ratios of a hydrophobic, a positively charged and a D-amino acid,

21. The non-hemolytic cytolytic random copolymer according to claim 20, composed of lysine, leucine and D-leucine in the ratio 1 : 1 : 1, 2 : 1 : 1 or 3 : 1 : 1 (Mol).

22. A pharmaceutical composition comprising a non-hemolytic cytolytic agent according to ^{claim 1} ~~any one of claims 1-21~~, and a pharmaceutically acceptable carrier.

23. The pharmaceutical composition according to claim 22, for the treatment of infections caused by pathogenic organisms.

24. The pharmaceutical composition according to claim 23, wherein the pathogenic organism is selected from bacteria, fungi, protozoa, mycoplasma and virus.

25. The pharmaceutical composition according to claim 24, wherein the pathogenic organism is a bacterium.

26. The pharmaceutical composition according to claim 22, for the treatment of cancer.

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